



LASERLAB-EUROPE

The Integrated Initiative of European Laser Research Infrastructures III

Grant Agreement number: 284464

Work package 30 – Laser and Photonics for Biology and Health (BIOPTICHAL)

Deliverable D30.11

Intermediate report on in vivo imaging techniques

Lead Beneficiary: 21 (University of Latvia)

Due date: month 12

Date of delivery: month 12

Project webpage: www.laserlab-europe.eu

<i>Deliverable Nature</i>	
R = Report, P = Prototype, D = Demonstrator, O = Other	R
<i>Dissemination Level</i>	
PU = Public PP = Restricted to other programme participants (incl. the Commission Services) RE = Restricted to a group specified by the consortium (incl. the Commission Services) CO = Confidential, only for members of the consortium (incl. the Commission Services)	PU

A. Abstract / Executive Summary

The method and workstation for parallel measuring of tissue fluorescence lifetimes (FLT) and photobleaching rates (PBR) has been developed and laboratory tested.

B. Deliverable Report

1 Introduction

The primary focus of this activity is development of workstation for parallel imaging of tissue FLT and PBR. To achieve this goal a picosecond laser system for FLT measurements has been assembled and laboratory tested. The main tasks of this period was assembling and adapting the FLT system for in-vivo measurements using fiber optic probe. This part of the study is important step for achieving the main goal - development a workstation for parallel imaging.

2 Objectives

The main task for Latvian group is development of workstation for parallel imaging of tissue fluorescence lifetimes and bleaching rates. It includes following sub-tasks:

1. Assembling and testing of a point monitoring fluorescence lifetime (FLT) system.
2. In-vitro and in-vivo measurements, data collection and analysis.
3. Parallel BPR and FLT in-vivo measurements, data collection and analysis.
4. Complex system ensuring parallel FLT and PBR imaging.
5. Prototype device for fluorescent skin diagnostics.

Currently (30/05/2013) the tasks 1-2 are completed; the tasks 3-5 is under development. The main achieved results are briefly described in this report.

3 Work performed / results / description

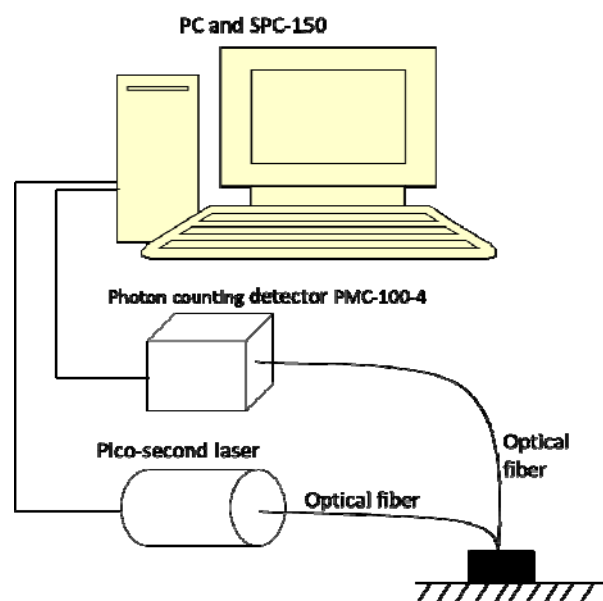
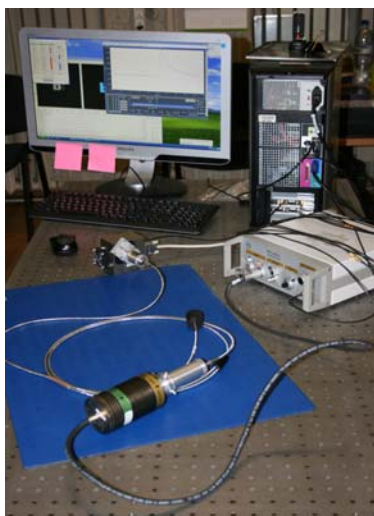


Fig.1. Workstation for parallel point measuring of tissue fluorescence lifetimes and photobleaching rates.

The scheme and photography of workstation for parallel measuring of tissue FLT and PBR is presented in fig.1. The setup consisted of picosecond lasers (405 nm, 470 nm, 510 nm), continuous lasers (405 nm, 473 nm, 532 nm), Y-shape fiber optic probe (6 x 400 μm for light delivery and 1 x 400 μm for registration), photon counter, synchronizer, in-line filter holder, computer with software.

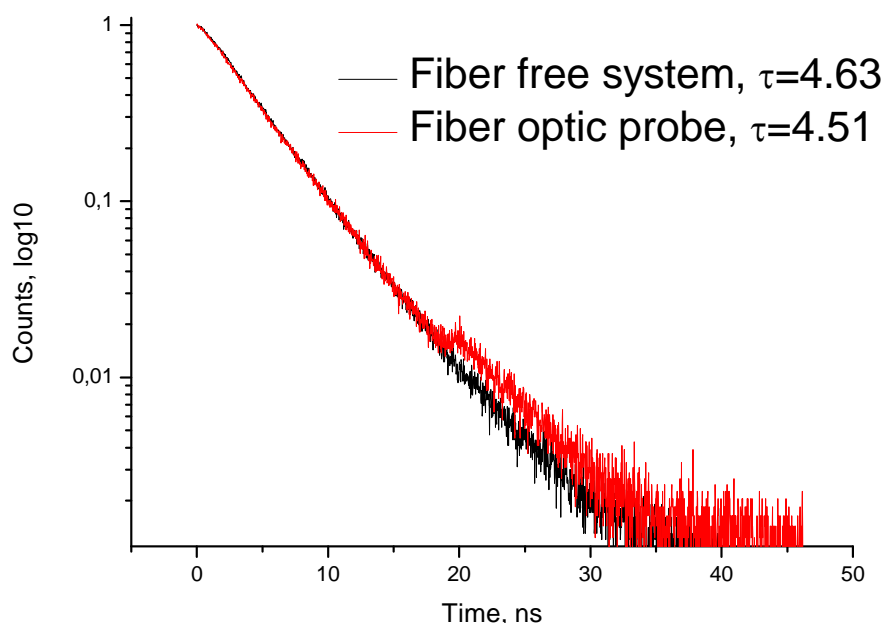


Fig.2. The effect of fiber optic probe on Chlorine e6 FLT kinetics.

Figure 2 illustrates the impact of fiber optic probe on the Chlorine e6 FLT kinetics. The impact of fiber optic probe manifested as some artifacts at 20 - 25 ns. These artifacts caused by light multiple scattering and reflections inside the fiber. Approximate impact on the FLT is not more than 3% that is quite reasonable.

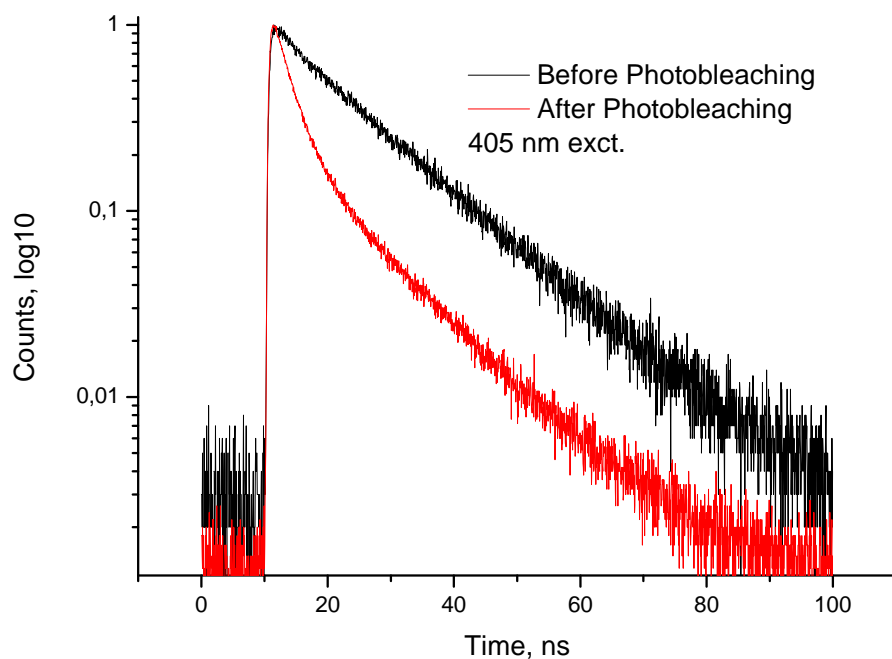


Fig.3. Florescence lifetime kinetics of protoporphyrin IX diluted in BSA before and after photobleaching

Protoporphyrin IX - BSA solution fluorescence lifetime changes after photobleaching is presented in figure 3. The solution was irradiated by 532 nm continuous laser during 10 minutes, with power density 50 mW/cm². As shown in figure, after photobleaching the kinetics undergoes the serious changes, respectively, appears additional decay component. The appearance of additional component indicates on changes in fluorophore composition changes. In this case, during the photobleaching new fluorophore was produced.

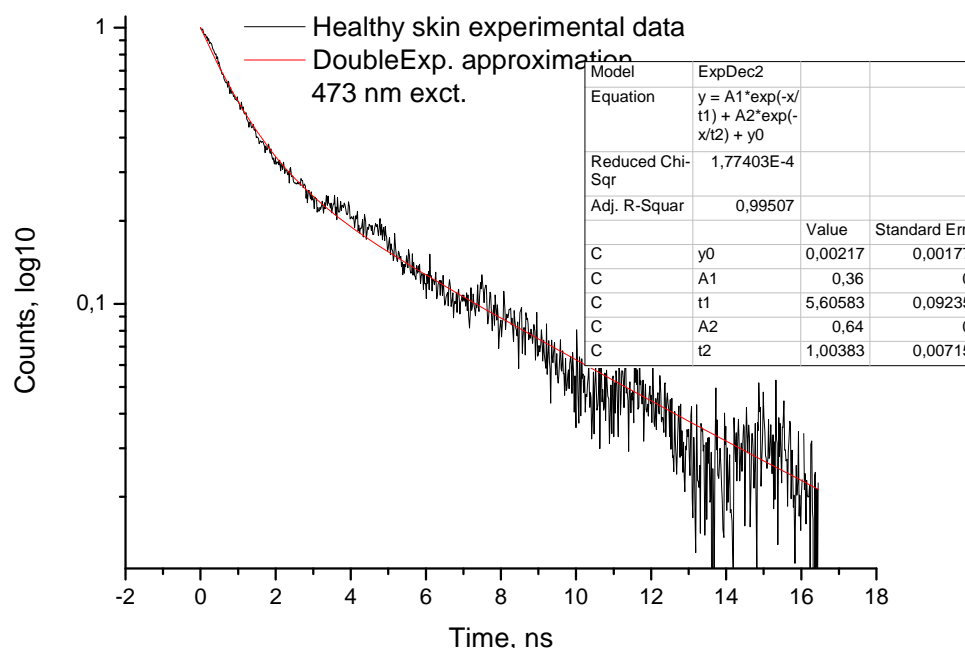


Fig.4. *In-vivo* healthy skin autofluorescence lifetime kinetics and its double exponential approximation.

In figure 4 is shown the first *in-vivo* FLT results. Kinetics was approximated by double exponential decay function. The fast component is $\tau_1 = 1$ ns, slow component $\tau_2 = 5.6$ ns. These results quite good correlate with the literature data. Obtained result demonstrates the ability of assembled system for measuring of autofluorescence lifetimes *in-vivo*.

4 Conclusions

- Assembled system is suitable for *in-vivo* FLT measurements.
- The fiber optic probe does not introduce the serious impact on the measured FLT.
- Experimentally demonstrated the FLT changes after the photobleaching.

5 References/Publications

1. I.Ferulova, A.Rieba, J.Lesins, A.Berzina, A.Lihachev, J.Spigulis. "Portable device for skin autofluorescence photobleaching measurements." *Lith. J. Phys.*, v 52(1), 55-58 (2012).
2. J.Spigulis. Biophotonic technologies for noninvasive assessment of skin condition and microcirculation, *Latv.J.Phys.Techn.Sci.*, in press (2012)
3. I.Ferulova, J.Lesins, A.Lihachev, D.Jakovels, J.Spigulis, Influence of low power CW laser irradiation on skin hemoglobin changes, *Proc. SPIE* 8427, 84273I (2012).